

Fungus Associated with a Granuloma in a Turkish Fish, *Aphanius chantrei* Gaillard

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(Plates I & II; Text-figures 1-11)

INTRODUCTION

RELATIVELY few fungus infections that produce tumors in fishes have been studied in detail. Montpellier & Dieugeide (1933) briefly described a "pseudo-tumeur mycélienne" in a North African *Cyprinodon fasciatus*, and recently Walker (1951) reported a case in *Salmo* sp. The present paper describes a granuloma caused by an undetermined fungus in *Aphanius chantrei*, a small, freshwater cyprinodont fish indigenous to Turkey.

Since the blood cells play an important role in the development of granulomas, it is fortunate that the papers by Duthie (1939) and Catton (1951) on fish blood elements are now available. This makes possible a more complete analysis of the piscine granuloma than has previously been presented.

The present study may have special significance in view of the recent investigations of Diller & Fisher (1950), who demonstrated the presence of inter- and intracellular fungal structure including mycelia (Fungi Imperfecti related to Blastomycetes) from transplanted, induced and spontaneous tumors of the mouse and of man.

MATERIALS AND METHODS

A number of *Aphanius chantrei* were collected near Samsun, a town in northern Anatolia on the Black Sea. They were kept for about a year in the aquarium of the Zoological Institute of the University of Istanbul at Baltalimani on

the Bosphorus. One of these specimens, a female 45 mm. in standard length, developed a tumor on the dorsal surface over the operculum and pectoral fins (Pl. I, Fig. 1). The swelling did not interfere with the normal behavior of the fish.

The fish was fixed in a mixture of 76 parts of saturated aqueous mercuric chloride, 20 parts of formalin and 4 parts of glacial acetic acid. The anterior half of the animal was decalcified in nitric acid, dehydrated in alcohol, cleared in xylol, embedded in paraffin and cross-sectioned at 10 μ . Sections were stained either with Mayer's hematoxylin (hemalum) and eosin, Heidenhain's iron hematoxylin and eosin, Mallory's azan stain or van Gieson's collagen stain, or by Gram's method.

GROSS DESCRIPTION OF THE TUMOR

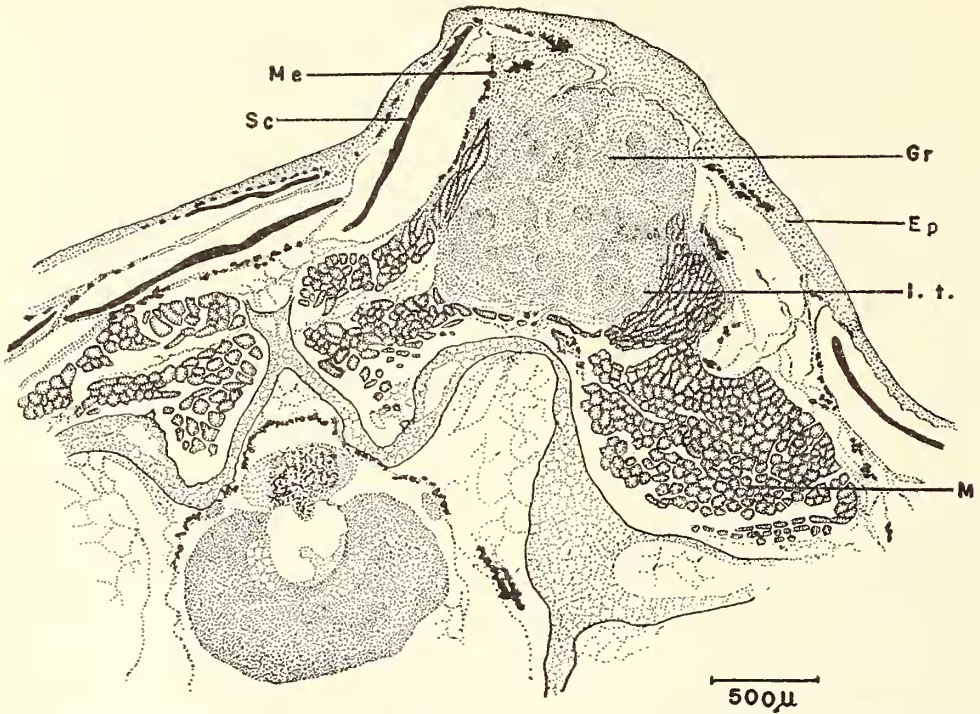
The swelling was smooth, soft, and projected dorsad from the right side of the body (Pl. I, Figs. 1-3; Text-figs. 1, 2). It measured 7 mm. in length, 5.5 mm. in depth and 4 mm. in height. The entire mass was covered by a layer of skin and was the same color as the body of the fish. The scales over the swelling were separated and no longer imbricated. Scales were missing on the right dorsal surface and in some other areas (Text-figs. 1, 2).

Examination of the sections showed that the growth was located in the dorsal musculature extending from the region just back of the head (Pl. I, Figs. 1, 2) to that near the gonads.

In some regions the skin in contact with the tumor was hyperplastic (Pl. I, Fig. 3; Text-fig. 1). In other regions the skin was normal and there was a space between the boundary of the tumor and the dermis.

At the most anterior portion of the growth there was no external swelling but the skin was hyperplastic and the scale pockets were larger

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TEXT-FIG. 1. A cross-section of the granuloma over the gill region. The new growth arises cone-like above the dorsal surface of the body. Note the hyperplastic epidermis on the right and the nodules in the granuloma. Ep = epidermis, Gr = granuloma, I.t. = infiltrating tissue, M = musculature, Me = melanophore, Sc = scale. Hemalum-eosin.

than those over the unaffected regions of the body. Here the growth, which contained some melanophores, had infiltrated the muscles between the skin and the skull.

A little further caudad the growth enlarged and extended above the body surface. The center of the growth contained nodule-like formations. On the right side of the mass, the epidermis and dermis of the skin were hyperplastic and extended into the growth. The scales and the scale pockets were wanting (Text-fig. 1). The left side was normal.

Still further caudad, in the gill region, the growth was larger and rounder. The overlying skin was thin and contained scales. The growth had infiltrated the dorsal trunk musculature of the right side of the body. Here the growth was composed primarily of whorl-like nodules which were surrounded by a fibrillar, capsule-like tissue.

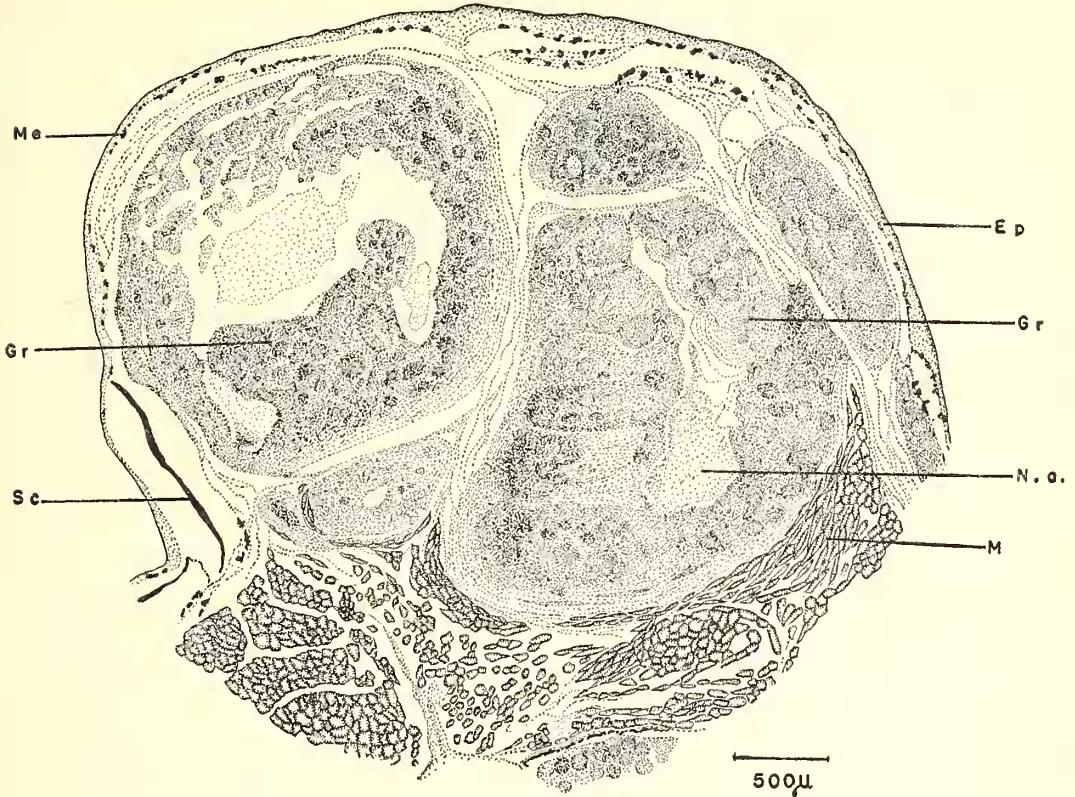
The largest part of the growth developed in the region above the liver (Text-fig. 2). The entire mass was round and was composed of two large and four small foci, each of which was

encapsulated. The centers of some of the foci were necrotic. At this level the infiltration of the muscles was seen only on the right side, but the pressure of the growth had forced the central muscle mass to the left. Posterior to this region, the tumor as a whole was smaller. Microscopic examination of the region just beyond the externally visible tumor revealed granulation tissue cells.

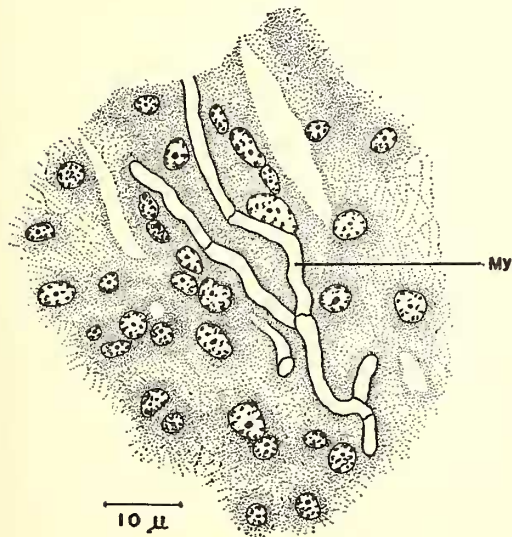
HISTOLOGICAL AND CYTOLOGICAL OBSERVATIONS

The granuloma is composed of a reticular connective tissue stroma and of proliferated lymph cells, leucocytes, mast cells, giant cells, fibroblasts and capillaries. No bacteria were found in sections stained by Gram's method.

The mycelia of an undetermined species of fungus are surrounded by lymphocytes, leucocytes and fibroblasts (Text-figs. 3-5). Most of the hyphae show septa and branches (Text-fig. 3; Pl. II, Fig. 4). The hyphae may be intracellular or intercellular. They lie either in granulation tissue as individual filaments or in an irregular network of mycelia (Text-figs. 3, 4). In hemalum-eosin-stained sections they were yellowish-pink in color; stained by Mallory's they are blue. All hyphae are Gram negative. They show a hyalin structure and homogeneous plasma. The width of the hyphae varies from 1.2 to 2.5 μ while their length varies approximately from 15 to 110 μ .



TEXT-FIG. 2. A cross-section in region of the liver showing different foci and necrotic area (N.a.) in the granuloma. Ep = epidermis, Gr = granuloma, M = musculature, Me = melanophore, Sc = scale. Hemalum-eosin.

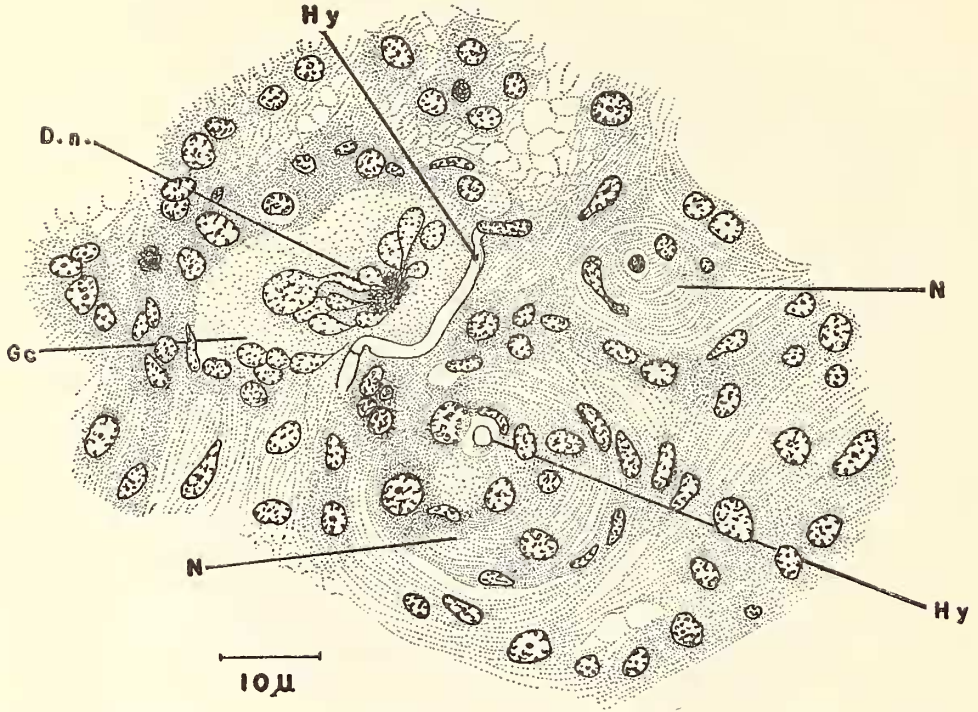


TEXT-FIG. 3. Part of a nodule containing a mycelium (My.). Note the septa in the hyphae. Hemalum-eosin.

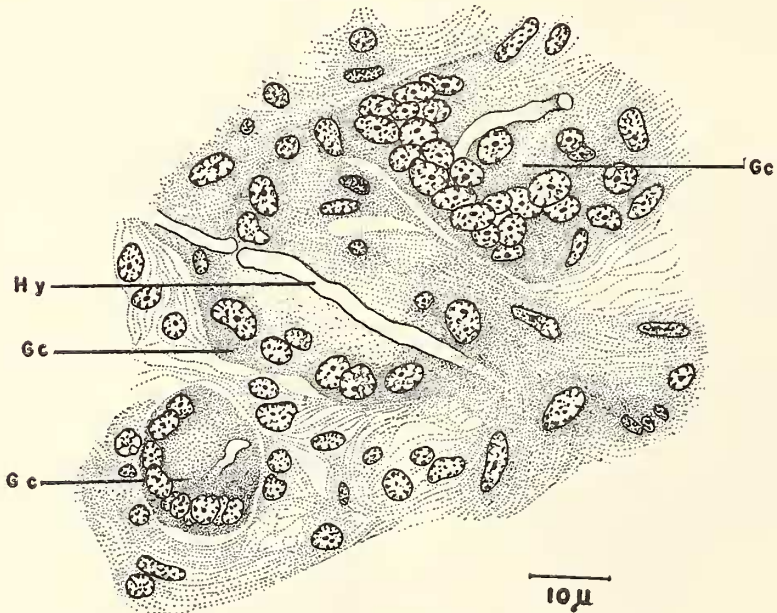
The fibers of the connective tissue stroma, in the center of the growth, are reticular and run approximately parallel around the whole granuloma (Text-figs. 6, 7). The mesenchyme cells or reticulum cells are 9 to 13 μ, spindle-shaped, and have elongated nuclei (3.5 to 13 μ) which contain granular chromatin. These cells are attached to the fibers.

Lymphocytes of different shapes and sizes, many of which show mitosis, are scattered between the reticular network. The majority of them are round, except those around the nodules which are elongate. These lymphocytes have a relatively thin plasma mass around their nuclei, which contain either granular chromatin or a chromatin network. They measure 6 to 9 μ. Their nuclei are 3.5 to 5.5 μ. These cells are characteristically found around mycelia (Text-figs. 6-8).

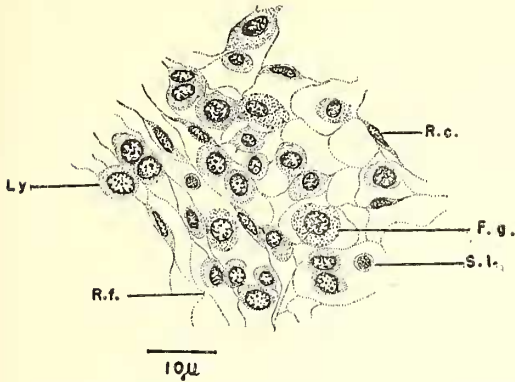
More finely granulated lymphocytes, called "fine granulocytes" by Duthie (1939) and Catton (1951), are found in the reticulum of the granuloma. These cells possess fine eosinophilic granules and measure 4.5 to 8.5 μ. Their nuclei, which measure 3 to 4.5 μ, contain a granular network in which the nucleoli can be seen in some instances. These fine granulocytes may be



TEXT-FIG. 4. Part of the granuloma showing a giant cell (G.c.) with dividing nuclei (D.n.). One of two small nodules (N) contains a hypha (Hy), seen in cross-section, and the other contains a degenerating cell. Hemalum-eosin.



TEXT-FIG. 5. Part of the granuloma showing three different giant cells (G.c.), each of which contains a hypha (Hy). Hemalum-eosin.



TEXT-FIG. 6. Reticular stroma of the granuloma showing reticular fibers (R.f.), reticulum cells (R.c.), lymphocytes (Ly), fine granulocytes (F.g.) and small lymphocytes (S.l.).

found either in capillaries or in the reticulum (Text-figs. 6-8). The fine granulocytes apparently pass through the blood vessels as shown in Text-fig. 8. Occasionally they may assume an elongated form, forming oval nuclei.

Some very small, round lymphocytes, having a diameter of 2.5 to 3.5 μ , are also seen, but rarely. These "small lymphocytes" have small amounts of cytoplasm around their nuclei. The nuclei, which are 1.5 to 2.5 μ in size, contain fine and extensive granular chromatin (Text-fig. 6, S.l.). These cells are similar to those called "lymphoid hemoblasts" by Duthie (1939) and "small lymphoid hemoblasts" by Catton (1951).

Another type of granuloma cell is represented by coarse granulocytes or mast cells. These are found mostly in the fibrillar, infiltrating, capsule-like zone around the granuloma (Text-fig. 9) and in the hyperplastic epidermis between the basal cells (Text-fig. 10). These mast cells or coarse granulocytes (10 to 20 μ) are spindle-shaped, round or oval and contain large eosinophilic granules. The nuclei are relatively small (2.5 to 3.5 μ). They are mostly eccentric and contain granular chromatin. These cells are found in the tissue of the new growth, but not in the blood vessels, nor are they found in the blood of a normal *Aphanius*.

The fibroblasts, which are elongated cells with oval or spindle-shaped nuclei, are scattered in the granuloma. They measure 13 to 23 μ and their nuclei, which very often contain a visible nucleolus, are 9.5 to 14.5 μ in size (Text-figs. 7-9). The fibroblasts take part in the infiltration of muscle and the formation of nodules in the granuloma.

Macrophages are found in necrotic areas. They are smaller than fibroblasts, similar to the

lymphocytes and difficult to distinguish from the latter.

Giant cells are distributed in the granulation tissue, particularly where the mycelia of fungus are scattered (Text-figs. 4, 5). They are polymorphic and contain round or oval nuclei which have nucleoli and loose chromatin nets. The nuclei are very often at the periphery of the cells and often form "pearls." The number of nuclei varies, many cells containing 10 to 27. The cells measure 19 to 40 μ and their nuclei, 3.2 to 8 μ . Text-fig. 5 shows three different types of giant cells embedded in granulation tissue, each of which has phagocytized one hypha.

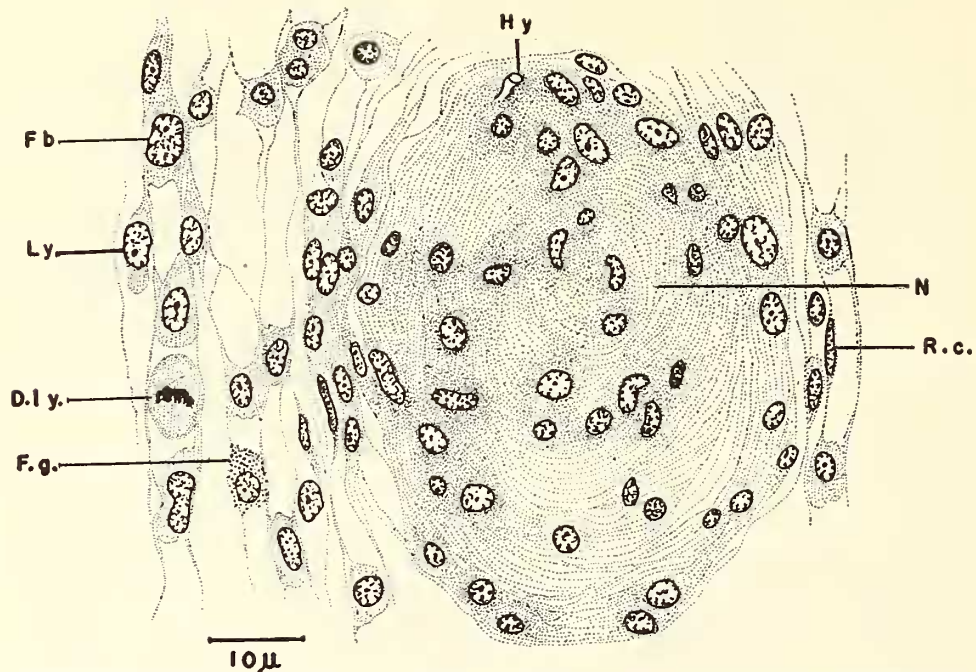
Whether giant cells are derived from fibroblasts or lymphocytes or endothelial cells has not been decided. Nuclear division was found in one of the giant cells. As shown in Text-fig. 4, the nuclear divisions are most peculiar. They probably represent a kind of amitotic division which may produce the pearl-like distribution of nuclei. Plehn (1906) found a similar type of nuclear division in a sarcoma in the trunk muscles of *Phoxinus laevis*. According to her, after such division multinuclear giant cells may appear. Some of the giant cells contain pyknotic nuclei.

The cells including lymphocytes and fibroblasts, which accumulate around the mycelia, form the nodules of granulation tissue (Pl. II, Figs. 5-6). In the formation of nodules lymphocytes are especially active (Text-figs. 7, 8). The nodules are round, vary in size, and are encapsulated by a reticular stroma, as shown in Text-fig. 7.

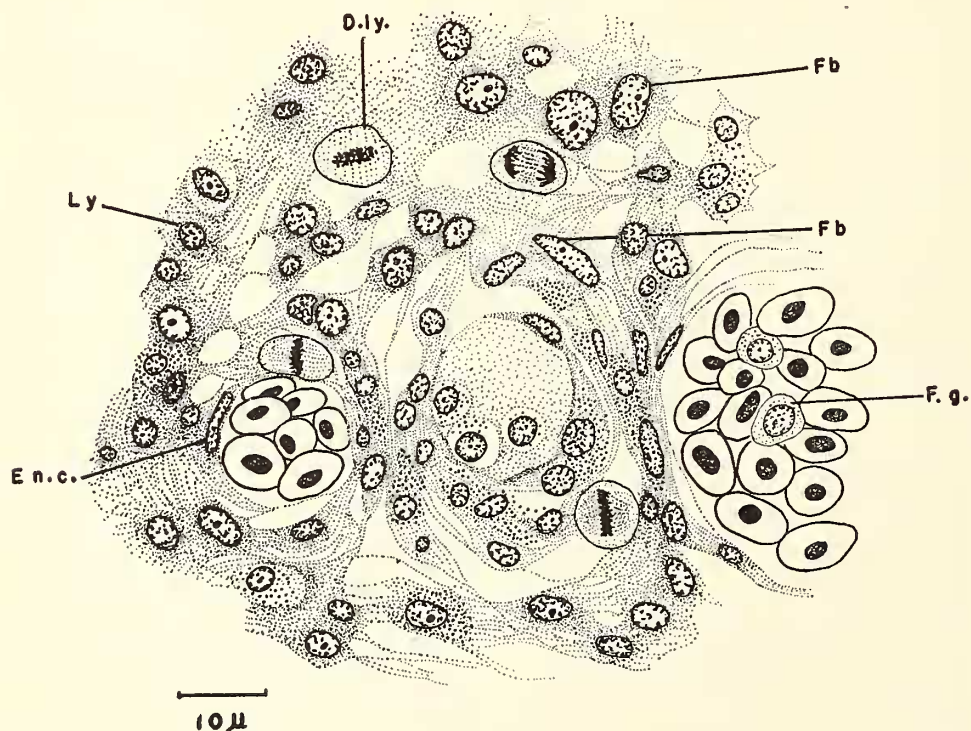
Some necrotic areas are found in the center of the granuloma. These areas contain cell debris, hyphae and macrophages (Pl. II, Fig. 5).

Capillaries are found either in or around the granuloma (Text-fig. 8). They are hyperemic and their endothelial cells are sometimes hypertrophic. Some erythrocytes lie free in the granulation tissue.

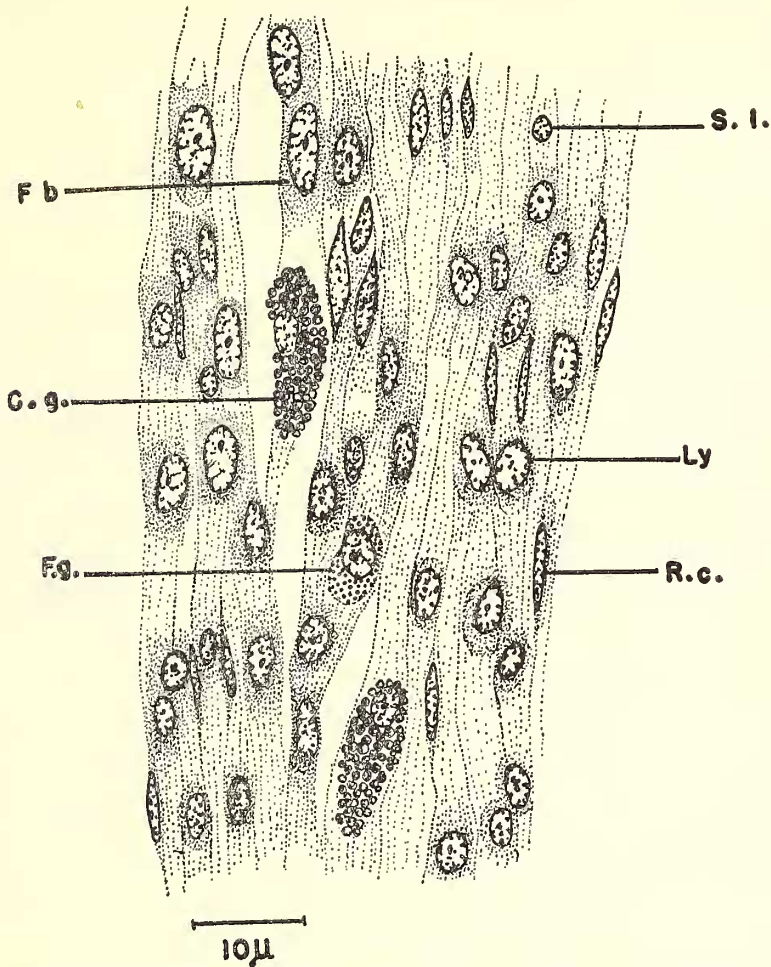
The epidermis over the granuloma is partly normal and partly hyperplastic. The normal epidermis of *Aphanius* is 20 to 30 μ thick and is composed of about six layers of squamous epithelium cells, oval or round mucous cells and basal cells, and some melanophores in the basal part of the epidermis. The hyperplastic epidermis over the granuloma is approximately 130 μ thick and has 20 to 25 cell layers (Text-fig. 10). Besides the squamous epithelial cells, mucous cells and basal cells, some coarse granulocytes are found in the proximal part of the hyperplastic epidermis. Since the coarse granulocytes in normal fish are only in connective tissue, they probably come from granulation



TEXT-FIG. 7. A nodule (N) in the granuloma, surrounded by fibrillar tissue. D.ly = dividing lymphocyte, F.g. = fine granulocyte, Fb = fibroblast, Hy = hypha, Ly = lymphocyte, R.c. = reticulum cell. Hemalum-eosin.



TEXT-FIG. 8. Part of the granulation tissue containing blood capillaries and dividing lymphocytes (D.ly). En.c. = endothelium cell, F.g. = fine granulocyte, Fb = fibroblasts, Ly = lymphocytes. Hemalum-eosin.



TEXT-FIG. 9. Part of the fibrillar tissue which surrounds the granuloma. C.g. = coarse granulocyte (mast cell), F.g. = fine granulocyte, Fb fibroblast, Ly = lymphocyte, R.c. = reticulum cell, S.l. = small lymphocyte. Hemalum-eosin.

tissue which in some regions is adjacent to the epidermis. The part lying between the squamous and basal cells of the hyperplastic epidermis is looser than in the normal epidermis. In some regions, the hyperplastic epidermis resembles granulation tissue in character and contains lymphocytes, fine granulocytes and hyphae of fungus besides epidermis cells. As the granuloma grew upwards, the overlying epidermis apparently became stretched. The epidermis thus grew parallel to the new growth.

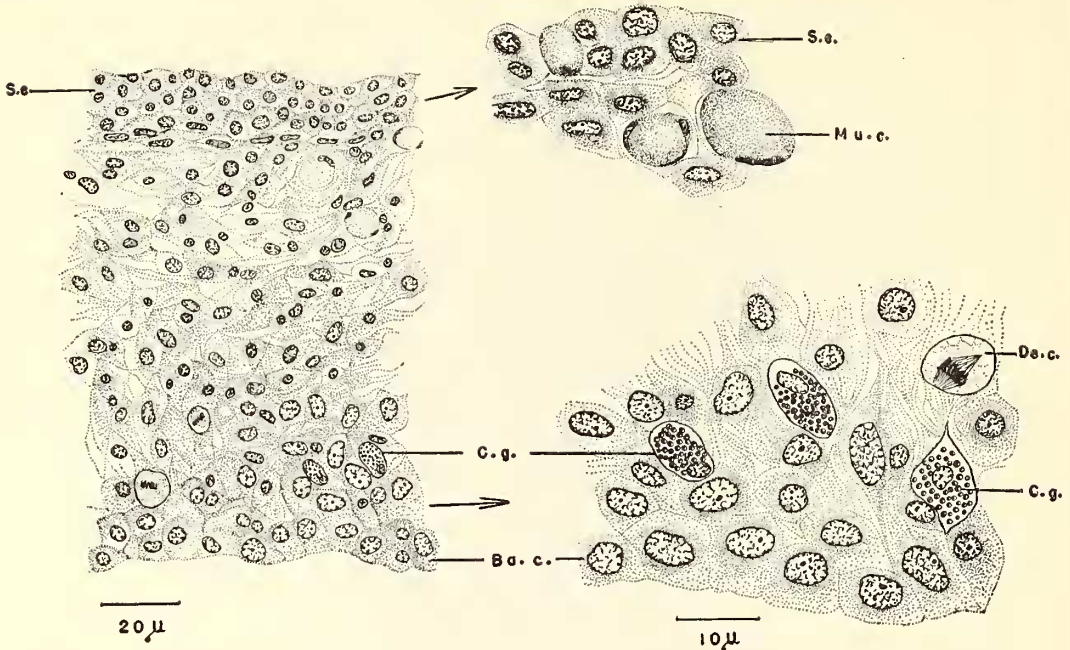
The scales are disarranged in some regions because of the epidermis growing under them, elevating them away from the dermis.

The fibrillar, capsule-like tissue which surrounds the granuloma infiltrates the subjacent musculature (Text-fig. 11; Pl. II, Fig. 7). At its deepest penetration, the invading granuloma cells appear fibrosarcoma-like and malignant.

DISCUSSION

In the granuloma described above, two phenomena must be distinguished. One is the cellular reaction of the body to the fungus infection. This results in the formation of granulation tissue in the infected area. Some of the cellular elements of granulation tissue, such as lymph cells, leucocytes and fibroblasts, form a nodule around either the mycelia or a single hyphae. The second phenomenon is the formation of a fibrosarcoma-like growth. The granulation tissue appears to lose its protective function and becomes a malignant growth. This tumor in *Aphanius* may be regarded as an "infective granuloma," as defined by Hamperl (1944) and Ogilvie (1947) in human pathology.

Human granulomas are essentially similar in cellular elements to those of fishes (Ash & Spitz, 1945; Ewing, 1940).



TEXT-FIG. 10. Hyperplastic epidermis overlying the granuloma. Ba.c. = basal cells, C.g. = coarse granuloocyte, De.c. = dividing epithelial cell, Mu.c. = mucous cell, S.e. = squamous epithelium. Hemalum-eosin.

The species of infectious fungus could not be identified. As Ash & Spitz (1945) have pointed out, "It is usually not possible to identify a fungus in a histologic section. . . . The spores and mycelia of different species are too much alike to permit classification solely on morphology." Since the hyphae found in *Aphanius* contain septa, it can be said that this fungus does not belong to the Saprolegniaceae (Plehn, 1912).

The following reactions to the fungus were seen in *Aphanius*: a hyperplasia of lymph cells and leucocytes and the formation of giant cells. Similar general responses were found by Montpellier & Dieugeide (1933) in *Cyprinodon*, and by Walker (1951) in *Salmo*.

Some specific inflammatory responses and the formation of granulation tissue in fishes are mentioned by Plehn & Mulsow (1911), Takahashi (1929), Thomas (1931), Plehn (1938), Drew (1910-13), Wurmbach (1951), Breider & Schmidt (1951), etc., but the authors give few, if any cytological and histological details. Plehn (1910) and Schäperclaus (1941) report that in a disease called "Drehkrankheit," a sporozöon, *Lentospora cerebralis*, causes a granuloma in the auditory organs of trout, destroying this organ.

With regard to the origin of coarse granulo-

cytes or mast cells, which are seen only rarely in the blood of fishes, several authors indicate that they come from the connective tissue. Michels (1923), studying a whitefish (*Leuciscus* sp.) and the carp (*Cyprinus carpio*), suggested this origin. Duthie (1939) and Catton (1951) have frequently observed these cells in connective tissue and rarely in the blood of a variety of teleost fishes. From my observations, the views of Michels seem valid.

SUMMARY

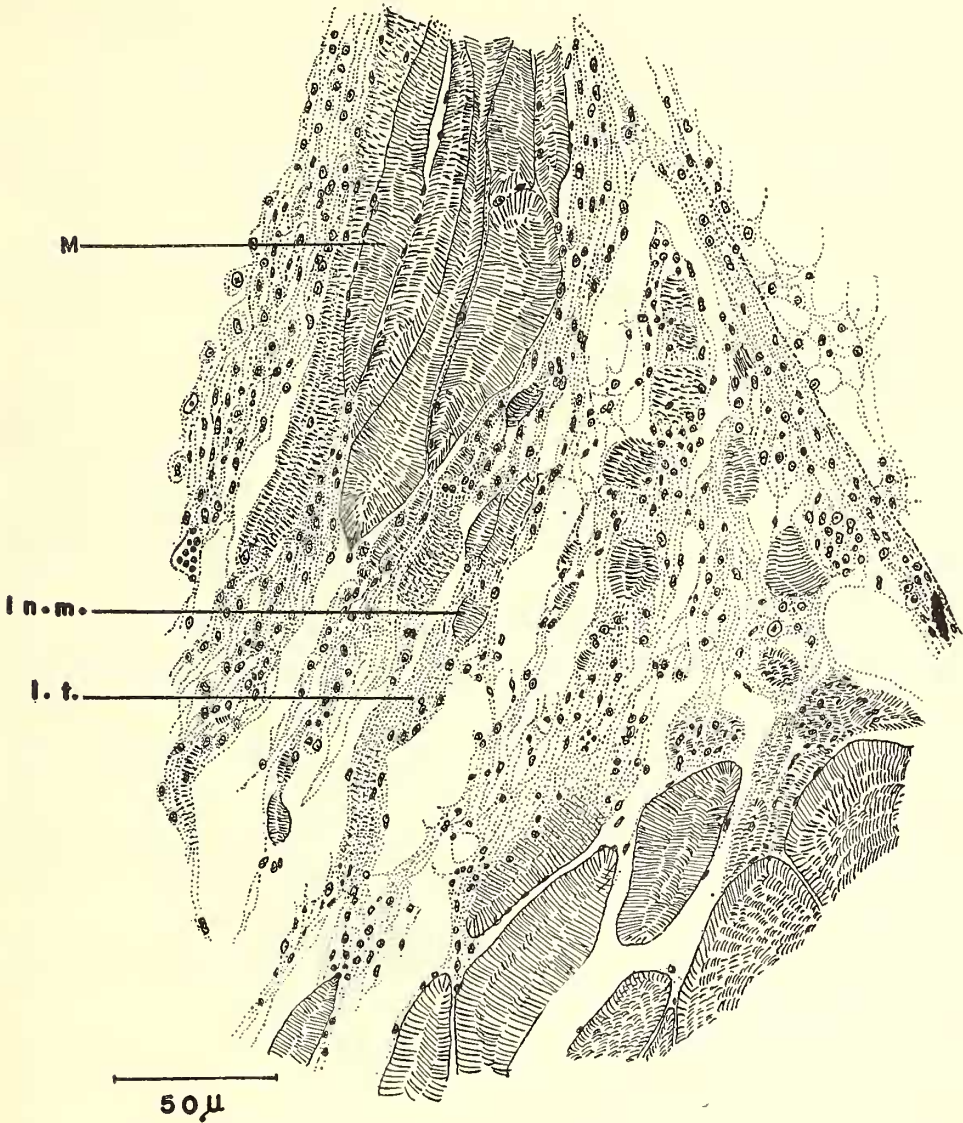
1. The histology and cytology of a granuloma associated with an undetermined fungus infection are described in a Turkish freshwater cyprinodont, *Aphanius chantrei*.

2. The granuloma mass, covered by a layer of skin, is found on the dorsal surface between the posterior margin of the operculum and the region of the pectoral fins.

3. The granuloma is composed of reticular connective tissue stroma and of proliferated lymph cells, leucocytes, mast cells, phagocytic giant cells, fibroblasts and macrophages. This mass is highly vascularized.

4. The mycelia of a fungus are scattered in the granulation tissue and are surrounded by lymphocytes and fibroblasts. These form definitive nodules in the granuloma.

5. The overlying epidermis, in some regions, is hyperplastic and shows partly the characteristics of granulation tissue.



TEXT-FIG. 11. Muscle infiltrating and fibrosarcoma-like tissue (I.t.). In.m. = infiltrated musculature, M = musculature. Hemalum-eosin.

6. The granuloma mass is surrounded by a fibrillar tissue which infiltrates the subjacent musculature. This infiltrating tissue is fibrosarcoma-like and appears to be malignant.

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EXPLANATION OF THE PLATES

(All photomicrographs were made from hemalum-eosin stained sections).

PLATE I

- Fig. 1. Granuloma on the dorsal surface in the region between the operculum and the pectoral fins of a female cyprinodont, *Aphanius chantrei*. About 1.4 \times .
- Fig. 2. Cross-section through the gill and operculum region showing the granuloma as a round, cone-like mass arising over the dorsal surface. 8.5 \times .
- Fig. 3. Another, more posterior, cross-section showing several foci of granuloma, one of which contains a large necrotic area. 8.5 \times .

PLATE II

- Fig. 4. Section showing two hyphae of a fungus in the granulation tissue. 1600 \times .
- Fig. 5. Section through one part of the granuloma showing many nodules. A necrotic area (gray) may be seen in lower left portion. 65 \times .
- Fig. 6. Section showing a part of the granuloma. Two large and some small nodules in the granulation tissue are shown at the lower right. Fibrillar and capsule-like tissue are at the lower left. These tissues are composed of fibroblasts, lymph cells and leucocytes, which infiltrate the musculature (extreme left). 375 \times .
- Fig. 7. The muscle infiltrated by fibrillar, fibrosarcoma-like tissue. Some muscle is not yet completely destroyed.